

human G-protein coupled receptor

Description

5 The present invention relates to novel identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to a G-protein coupled receptor (GPCR), hereinafter referred to as IGS3. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides, to a vector containing said
10 polynucleotides, a host cell containing such vector and transgenic animals where the IGS3-gene is either overexpressed, misexpressed, underexpressed and/or suppressed (knock-out animals). The invention further relates to a method for screening compounds capable to act as an agonist or an antagonist of said G-protein coupled receptor IGS3.

15 **BACKGROUND OF THE INVENTION**

 It is well established that many medically significant biological processes are mediated by proteins participating in signal transduction pathways that involve G-proteins and/or second messengers; e.g., cAMP (Lefkowitz, Nature, 1991, 351:353-354). Herein these proteins are
20 referred to as proteins participating in pathways with G-proteins. Some examples of these proteins include the GPC receptors, such as those for adrenergic agents and dopamine (Kobilka, B.K., et al., Proc. Natl. Acad. Sci., USA, 1987, 84:46-50; Kobilka, B.K., et al., Science, 1987, 238:650-656; Bunzow, J.R., et al., Nature, 1988, 336:783-787), G-proteins themselves, effector proteins, e.g., phospholipase C, adenylate cyclase, and phosphodiesterase, and
25 actuator proteins, e.g., protein kinase A and protein kinase C (Simon, M.I., et al., Science, 1991, 252:802-8).

 For example, in one form of signal transduction, upon hormone binding to a GPCR the receptor interacts with the heterotrimeric G-protein and induces the dissociation of GDP from the
30 guanine nucleotide-binding site. At normal cellular concentrations of guanine nucleotides, GTP fills the site immediately. Binding of GTP to the α subunit of the G-protein causes the dissociation of the G-protein from the receptor and the dissociation of the G-protein into α and $\beta\gamma$ subunits. The GTP-carrying form then binds to activated adenylate cyclase. Hydrolysis of GTP to GDP, catalyzed by the G-protein itself (α subunit possesses an intrinsic GTPase activity),
35 returns the G-protein to its basal, inactive form. The GTPase activity of the α subunit is, in essence, an internal clock that controls an on/off switch. The GDP bound form of the α subunit has high affinity for $\beta\gamma$ and subsequent reassociation of α GDP with $\beta\gamma$ returns the system to the

basal state. Thus the G-protein serves a dual role, as an intermediate that relays the signal from receptor to effector (in this example adenylate cyclase), and as a clock that controls the duration of the signal.

5 The membrane bound superfamily of G-protein coupled receptors has been characterized as having seven putative transmembrane domains. The domains are believed to represent transmembrane α -helices connected by extracellular or cytoplasmic loops. G-protein coupled receptors include a wide range of biologically active receptors, such as hormone, viral, growth factor and neuroreceptors.

10 The G-protein coupled receptor family includes dopamine receptors which bind to neuroleptic drugs used for treating CNS disorders. Other examples of members of this family include, but are not limited to calcitonin, adrenergic, neuropeptideY, somastotatin, neurotensin, neurokinin, capsaicin, VIP, CGRP, CRF, CCK, bradykinin, galanin, motilin, nociceptin,
15 endothelin, cAMP, adenosine, muscarinic, acetylcholine, serotonin, histamine, thrombin, kinin, follicle stimulating hormone, opsin, endothelial differentiation gene-1, rhodopsin, odorant, and cytomegalovirus receptors.

20 Most G-protein coupled receptors have single conserved cysteine residues in each of the first two extracellular loops which form disulfide bonds that are believed to stabilize functional protein structures. The 7 transmembrane regions are designated as TM1, TM2, TM3, TM4, TM5, TM6 and TM7. The cytoplasmic loop which connects TM5 and TM6 may be a major component of the G-protein binding domain.

25 Most G-protein coupled receptors contain potential phosphorylation sites within the third cytoplasmic loop and/or the carboxy terminus. For several G-protein coupled receptors, such as the β -adrenoreceptor, phosphorylation by protein kinase A and/or specific receptor kinases mediates receptor desensitization.

30 Recently, it was discovered that certain GPCRs, like the calcitonin-receptor like receptor, might interact with small single pass membrane proteins called receptor activity modifying proteins (RAMP's). This interaction of the GPCR with a certain RAMP is determining which natural ligands have relevant affinity for the GPCR-RAMP combination and regulate the functional signaling activity of the complex (McLathie, L.M. et al., Nature (1998) 393:333-339).

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For some receptors, the ligand binding sites of G-protein coupled receptors are believed to comprise hydrophilic sockets formed by several G-protein coupled receptor transmembrane domains, said sockets being surrounded by hydrophobic residues of the G-protein coupled receptors. The hydrophilic side of each G-protein coupled receptor transmembrane helix is postulated to face inward and form a polar ligand-binding site. TM3 has been implicated in several G-protein coupled receptors as having a ligand-binding site, such as the TM3 aspartate residue. TM5 serines, a TM6 asparagine and TM6 or TM7 phenylalanines or tyrosines are also implicated in ligand binding.

G-protein coupled receptors can be intracellularly coupled by heterotrimeric G-proteins to various intracellular enzymes, ion channels and transporters (see, Johnson et al., Endoc. Rev., 1989, 10:317-331). Different G-protein α -subunits preferentially stimulate particular effectors to modulate various biological functions in a cell. Phosphorylation of cytoplasmic residues of G-protein coupled receptors has been identified as an important mechanism for the regulation of G-protein coupling of some G-protein coupled receptors. G-protein coupled receptors are found in numerous sites within a mammalian host.

Receptors - primarily the GPCR class - have led to more than half of the currently known drugs (Drews, Nature Biotechnology, 1996, 14: 1516). This indicates that these receptors have an established, proven history as therapeutic targets. The new IGS3 GPCR described in this invention clearly satisfies a need in the art for identification and characterization of further receptors that can play a role in diagnosing, preventing, ameliorating or correcting dysfunctions, disorders, or diseases, hereafter generally referred to as "the Diseases". The Diseases include, but are not limited to, psychiatric and CNS disorders, including schizophrenia, episodic paroxysmal anxiety (EPA) disorders such as obsessive compulsive disorder (OCD), post traumatic stress disorder (PTSD), phobia and panic, major depressive disorder, bipolar disorder, Parkinson's disease, general anxiety disorder, autism, delirium, multiple sclerosis, Alzheimer disease/dementia and other neurodegenerative diseases, severe mental retardation, dyskinesias, Huntington's disease, Tourett's syndrome, tics, tremor, dystonia, spasms, anorexia, bulimia, stroke, addiction/dependency/craving, sleep disorder, epilepsy, migraine; attention deficit/hyperactivity disorder (ADHD); cardiovascular diseases, including heart failure, angina pectoris, arrhythmias, myocardial infarction, cardiac hypertrophy, hypotension, hypertension - e.g. essential hypertension, renal hypertension, or pulmonary hypertension, thrombosis, arteriosclerosis, cerebral vasospasm, subarachnoid hemorrhage, cerebral ischemia, cerebral infarction, peripheral vascular disease, Raynaud's disease, kidney disease - e.g. renal failure; dyslipidemias; obesity; emesis; gastrointestinal disorders, including irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), gastroesophageal reflux disease (GERD), motility

disorders and conditions of delayed gastric emptying, such as post operative or diabetic gastroparesis, and diabetes, ulcers – e.g. gastric ulcer; diarrhoea; other diseases including osteoporosis; inflammations; infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; chemotherapy induced injury; tumor invasion; immune disorders; urinary retention; asthma; allergies; arthritis; benign prostatic hypertrophy; endotoxin shock; sepsis; complication of diabetes mellitus; and gynaecological disorders.

SUMMARY OF THE INVENTION

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In one aspect, the invention relates to IGS3 polypeptides, polynucleotides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such IGS3 polypeptides, polynucleotides and recombinant materials. Such uses include, but are not limited to, use as a therapeutic target and for treatment of one of the Diseases as mentioned above.

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In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with IGS3 imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate IGS3 activity or levels. A further aspect of the invention relates to animal-based systems which act as models for disorders arising from aberrant expression or activity of IGS3.

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BRIEF DESCRIPTION OF THE FIGURE

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Figure 1. Schematic representation of the relative positions of the different DNA clones that were isolated to generate the consensus IGS3 cDNA sequence. HNT1370 represents the "founding" genomic clone. λ -IGS3.1A,B etc. indicate separate (nearly) overlapping sequence contigs obtained from sequence analysis of DNA from lambda clone IGS3.1. PCR primers that have been described in this document are indicated (IP#). CONSENSUS denotes the contig that was obtained after merging all obtained sequences. The part of the CONSENSUS contig that was fully validated by sequence analysis of at least three independent clones is represented by IGS3DNA (SEQ ID NO: 1). The 330 amino acids long open reading frame present in IGS3DNA is indicated with "***". The position of EST AF003828 is indicated with "==".

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Table 1: IGS3-DNA of SEQ ID NO: 1

	5' -
5	TTAATCTCTTCAAGCCTCTGATTTCCTCTCCTGTAAACAGGGGCGGTAATTACCACATA ACAGGCTGGTCATGAAAATCAGTGAACATGCAGCAGGTGCTCAAGTCTTGTGTTTTGTTTC CAGGGGCACCAGTGGAGGTTTTCTGAGCATGGATCCAACCACCCCGGCCTGGGGAACAGA AAGTACAACAGTGAATGGAAATGACCAAGCCCTTCTTCTGCTTTGTGGCAAGGAGACCCT GATCCCGGTCTTCCTGATCCTTTTCATTGCCCTGGTCGGGCTGGTAGGAAACGGGTTTGT GCTCTGGCTCCTGGGCTTCCGCATGCGCAGGAACGCCTTCTCTGTCTACGTCCTCAGCCT 10 GGCCGGGGCCGACTTCTCTTCTCTGCTTCCAGATTATAAATTGCCTGGTGTACCTCAG TAACTTCTTCTGTTCCATCTCCATCAATTTCCCTAGCTTCTTCACCACTGTGATGACCTG TGCCTACCTTGAGGCCTGAGCATGCTGAGCACCGTCAGCACCGAGCGCTGCCTGTCCGT CCTGTGGCCCATCTGGTATCGCTGCCGCCGCCCCAGACACCTGTCAGCGGTCGTGTGTGT CCTGCTCTGGGCCCCTGTCCCTACTGCTGAGCATCTTGGAAGGGAAGTTCTGTGGCTTCTT 15 ATTTAGTGATGGTGA CTCTGGTTGGTGT CAGACATTTGATTT CATCACTGCAGCGTGGCT GATTTTTTTTATTCATGGTTCTCTGTGGGTCCAGTCTGGCCCTGCTGGTCAGGATCCTCTG TGGCTCCAGGGGTCTGCCACTGACCAGGCTGTACCTGACCATCCTGCTCACAGTGCTGGT GTTCTCCTCTGCGGCCTGCCCTTTGGCATT CAGTGGTT CCTAATATTATGGATCTGGAA GGATTCTGATGTCTTATTTTGT CATATT CATCCAGTTT CAGTTGTCCTGTCATCTCTTAA 20 CAGCAGTGCCAACCCCATCATTTACTTCTTCGTGGGCTCTTTTAGGAAGCAGTGGCGGCT GCAGCAGCCGATCCTCAAGCTGGCTCTCCAGAGGGCTCTGCAGGACATTGCTGAGGTGGA TCACAGTGAAGGATGCTTCCGT CAGGGCACCCCGAGATGTCGAGAAGCAGTCTGGTGTA GAGATGGACAGCCTCTACTTCCATCAGATATATGTG - 3'

Table 2: IGS3-protein of SEQ ID NO: 2

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MDPTTPAWGTESTTVNGNDQALLLLCGKETLIPVFLILFIALVGLVGNGFVLWLLGFRMR
RNAFSVYVLSLAGADFLFLCFQIINCLVYLSNFFCSISINFPSFFTTVMTCAYLAGLSML
STVSTERCLSVLWPIWYRCRRPRHLSAVVCVLLWALSLLLSILEGKFCGFLFSDGDSGWC
QTFDFITAAWLIFLFMVLCGSSLALLVRILCGSRGLPLTRLYLTILLTVLVFLLCGLPFG
IQWFLILWIWKDSDVLFCHIHVPSVVLSSLNSSANPIIYFFVGSFRKQWRLQQPILKLAL
QRALQDIAEVDHSEGCFRQGTPEMSRSSLV

DESCRIPTION OF THE INVENTION

Structural and chemical similarity, in the context of sequences and motifs, exists among the IGS3 GPCR of the invention and other human GPCR's. Therefore, IGS3 is implied to play a
5 role among other things in the Diseases mentioned above.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein
10 can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

15 Definitions

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

20 "IGS3" refers, among others, to a polypeptide comprising the amino acid sequence set forth in SEQ ID NO:2, or a Variant thereof.

"Receptor Activity" or "Biological Activity of the Receptor" refers to the metabolic or physiologic function of said IGS3 including similar activities or improved activities or these
25 activities with decreased undesirable side effects. Also included are antigenic and immunogenic activities of said IGS3.

"IGS3-gene" refers to a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO:1 or Variants thereof and/or their complements.
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"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of a Fab or other immunoglobulin expression library.

35 "Isolated" means altered "by the hand of man" from the natural state and/or separated from the natural environment. Thus, if an "isolated" composition or substance that occurs in nature has been "isolated", it has been changed or removed from its original environment, or

both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

5 "Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is a mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-
10 stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" may also include triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as
15 inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

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"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins, and/or to combinations thereof. Polypeptides
25 may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well-described in basic texts and in more detailed monographs, as well as in voluminous research literature. Modifications can occur anywhere in a polypeptide, including the
30 peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result
35 from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide

derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol; cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter et al., "Analysis for protein modifications and nonprotein cofactors", Meth. Enzymol. (1990) 182:626-646 and Rattan et al., "Protein Synthesis: Posttranslational Modifications and Aging", Ann. NY Acad. Sci. (1992) 663:48-62.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties such as essential biological, structural, regulatory or biochemical properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, and deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" per se has an art-recognized meaning and can be calculated using published techniques. See, e.g.: (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed.; Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I,

Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., SIAM J. Applied Math. (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., SIAM J. Applied Math. (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCG program package (Devereux, J., et al., Nucleic Acids Research (1984) 12(1):387), BLASTP, BLASTN, FASTA (Atschul, S.F. et al., J. Molec. Biol. (1990) 215:403). The word "homology" may substitute for the words "identity".

As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence of SEQ ID NO: 1 is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five nucleotide differences per each 100 nucleotides of the reference nucleotide sequence of SEQ ID NO: 1. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to any 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to any 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence, or in a number of nucleotides of up to any 5% of the total nucleotides in the reference sequence there may be a combination of deletion, insertion and substitution. These mutations of the reference sequence may occur at the 5 or 3 terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

Similarly, by a polypeptide having an amino acid sequence having at least, for example, 95% "identity" to a reference amino acid sequence of SEQ ID NO:2 is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of SEQ ID NO: 2. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to any 5% of the amino acid residues in the reference sequence may be deleted or substituted with another

amino acid, or a number of amino acids up to any 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either
5 individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

Polypeptides of the Invention

10 In one aspect, the present invention relates to IGS3 polypeptides (including IGS3 proteins). The IGS3 polypeptides include the polypeptide of SEQ ID NO:2 and the polypeptide having the amino acid sequence encoded by the DNA insert contained in the deposit no. CBS 102196, deposited on September 15, 1999 at the Centraalbureau voor Schimmelcultures at Baarn (The Netherlands); as well as polypeptides comprising the amino acid sequence of SEQ
15 ID NO:2 and the polypeptide having the amino acid sequence encoded by the DNA insert contained in the deposit no. CBS 102196 at the Centraalbureau voor Schimmelcultures at Baarn (The Netherlands), and polypeptides comprising an amino acid sequence having at least 80% identity to that of SEQ ID NO:2 and/or to the polypeptide having the amino acid sequence encoded by the DNA insert contained in the deposit no. CBS 102196 at the Centraalbureau voor
20 Schimmelcultures at Baarn (The Netherlands) over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to said amino acid sequence. Furthermore, those with at least 97%, in particular at least 99%, are highly preferred. Also included within IGS3 polypeptides are polypeptides having the amino acid sequence which has at least 80% identity to the polypeptide having the amino acid sequence of SEQ ID NO: 2 or
25 the polypeptide having the amino acid sequence encoded by the DNA insert contained in the deposit no. CBS 102196 at the Centraalbureau voor Schimmelcultures at Baarn (The Netherlands) over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97%, in particular at least 99% are highly preferred. Preferably IGS3 polypeptides exhibit at least one
30 biological activity of the receptor.

- In an additional embodiment of the invention, the IGS3 polypeptides may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which
35 aid in purification such as multiple histidine residues, sequences which aid in detection such as antigenic peptide tags (such as the haemagglutinin (HA) tag), or an additional sequence for stability during recombinant production.

Fragments of the IGS3 polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that is the same as part of, but not all of, the amino acid sequence of the aforementioned IGS3 polypeptides. As with IGS3 polypeptides, fragments
5 may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20; 21-40, 41-60, 61-80, 81-100; and 101 to the end of IGS3 polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at
10 either extreme or at both extremes.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of IGS3 polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl
15 terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta
20 amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments. Biologically active fragments are those that mediate receptor activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

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Thus, the polypeptides of the invention include polypeptides having an amino acid sequence that is at least 80% identical to either that of SEQ ID NO:2 and/or the polypeptide having the amino acid sequence encoded by the DNA insert contained in the deposit no. CBS 102196 at the Centraalbureau voor Schimmelcultures at Baarn (The Netherlands), or fragments
30 thereof with at least 80% identity to the corresponding fragment. Preferably, all of these polypeptide fragments retain the biological activity of the receptor, including antigenic activity. Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions – i.e., those that substitute a residue with another of like characteristics. Typical
35 such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or

aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

5 The IGS3 polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Methods for preparing such polypeptides are well known in the art.

Polynucleotides of the Invention

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A further aspect of the invention relates to IGS3 polynucleotides. IGS3 polynucleotides include isolated polynucleotides which encode the IGS3 polypeptides and fragments, and polynucleotides closely related thereto. More specifically, the IGS3 polynucleotide of the invention includes a polynucleotide comprising the nucleotide sequence contained in SEQ ID
15 NO:1, such as the one capable of encoding a IGS3 polypeptide of SEQ ID NO: 2, polynucleotides having the particular sequence of SEQ ID NO: 1 and polynucleotides which essentially correspond to the DNA insert contained in the deposit no. CBS 102196 at the Centraalbureau voor Schimmelcultures at Baarn (The Netherlands).

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IGS3 polynucleotides further include polynucleotides comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the IGS3 polypeptide of SEQ ID NO:2, polynucleotides comprising a nucleotide sequence that is at least 80% identical to that of SEQ ID NO:1 over its entire length and a polynucleotide which essentially corresponds to the DNA insert contained in the deposit no. CBS 102196 at the
25 Centraalbureau voor Schimmelcultures at Baarn (The Netherlands).

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In this regard, polynucleotides with at least 90% identity are particularly preferred, and those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred. Also included under IGS3 polynucleotides are a nucleotide sequence which has
30 sufficient identity to a nucleotide sequence contained in SEQ ID NO: 1 or to the DNA insert contained in the deposit no. CBS 102196 at the Centraalbureau voor Schimmelcultures at Baarn (The Netherlands) to hybridize under conditions useable for amplification or for use as a probe or marker. The invention also provides polynucleotides which are complementary to such IGS3 polynucleotides.

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IGS3 of the invention is structurally related to other proteins of the G-protein coupled receptor family, as shown by the results of BLAST searches in the public databases. The amino

acid sequence of Table 2 (SEQ ID NO:2) has about 35 % identity (using BLAST, Altschul S.F. et al. Nucleic Acids Res. (1997) 25:3389-3402) over most of its length (amino acid residues 2-306) with the protein encoded by the human mas oncogene (Sequence 1 in patent application WO 8707472). The sequence is 37% identical (amino acid residues 35-315) with the G-protein
5 coupled receptor published in patent application WO 9616087 (GENESEQ 96P-R97222). The nucleotide sequence of Table 1 (SEQ ID NO: 1) has 52 % and 54 % identity over most of its length to the two receptors above (GENESEQ 87N-70685 and 96N-T28807 respectively). Also there is 48% identity to the human Somatostatin-3 receptor in residues 104-1144 (WO 9313130; 93N-Q45657). Hydropathy analysis (Kyte J. et al., J. Mol. Biol. (1982) 157: 105-132; Klein P. et al., Biochim. Biophys. Acta (1985) 815: 468-476) of the IGS3 protein sequence expectedly
10 showed the presence of 7 transmembrane domains. Thus, IGS3 polypeptides and polynucleotides of the present invention are expected to have, inter alia, similar biological functions/properties to their homologous polypeptides and polynucleotides, and their utility is obvious to anyone skilled in the art.

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Polynucleotides of the invention can be obtained from natural sources such as genomic DNA. In particular, degenerated PCR primers can be designed that encode conserved regions within a particular GPCR gene subfamily. PCR amplification reactions on genomic DNA or cDNA using the degenerate primers will result in the amplification of several members (both known and
20 novel) of the gene family under consideration (the degenerated primers must be located within the same exon, when a genomic template is used). (Libert et al., Science, 1989, 244: 569-572). Polynucleotides of the invention can also be synthesized using well-known and commercially available techniques.

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The nucleotide sequence encoding the IGS3 polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in SEQ ID NO:1 (nucleotide number 149 to 1138), or it may be a different nucleotide sequence, which as a result of the redundancy (degeneracy) of the genetic code might also show alterations compared to the polypeptide encoding sequence contained in SEQ ID NO:1, but also encodes the polypeptide of SEQ ID
30 NO:2.

When the polynucleotides of the invention are used for the recombinant production of the IGS3 polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature polypeptide or
35 fragment in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions.

For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz et al., Proc. Natl. Acad. Sci USA (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further preferred embodiments are polynucleotides encoding IGS3 variants comprising the amino acid sequence of the IGS3 polypeptide of SEQ ID NO:2 in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination.

The polynucleotides of the invention can be engineered using methods generally known in the art in order to alter IGS3-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create amino acid substitutions, create new restriction sites, alter modification (e.g. glycosylation or phosphorylation) patterns, change codon preference, produce splice variants, and so forth.

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the polynucleotides described above. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 80%, and preferably at least 90%, and more preferably at least 95%, yet even more preferably at least 97%, in particular at least 99% identity between the sequences.

Polynucleotides of the invention, which are identical or sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1 or a fragment thereof, may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding IGS3 and to isolate cDNA and genomic clones of other genes (including genes encoding homologs and-orthologs from species other than human) that have a high sequence similarity to the IGS3 gene. People skilled in the art are well aware of such hybridization techniques. Typically these nucleotide sequences are 80% identical, preferably 90% identical, more preferably 95% identical to that of the referent. The probes generally will comprise at least 5 nucleotides, and preferably at least 8 nucleotides, and more preferably at least 10 nucleotides, yet even more preferably at least 12 nucleotides, in particular at least 15 nucleotides. Most preferred, such probes will have

at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

One embodiment, to obtain a polynucleotide encoding the IGS3 polypeptide, including
5 homologs and orthologs from species other than human, comprises the steps of screening an appropriate library under stringent hybridization conditions with a labeled probe having the SEQ ID NO: 1 or a fragment thereof, and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to those of skill in the art. Stringent hybridization conditions are as defined above or alternatively conditions under
10 overnight incubation at 42 °C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1xSSC at about 65°C.

15 The polynucleotides and polypeptides of the present invention may be used as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

Vectors, Host Cells, Expression

20

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be used to produce such proteins using RNAs
25 derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard
30 laboratory manuals, such as Davis et al., BASIC METHODS IN MOLECULAR BIOLOGY (1986) and Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or
35 infection.

Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, E. coli, Streptomyces and Bacillus subtilis cells; fungal cells, such as yeast cells and Aspergillus cells; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook et al., MOLECULAR CLONING, A LABORATORY MANUAL (supra).

20

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals, i.e. derived from a different species.

25

If the IGS3 polypeptide is to be expressed for use in screening assays, generally, it is preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. In case the affinity or functional activity of the IGS3 polypeptide is modified by receptor activity modifying proteins (RAMP), coexpression of the relevant RAMP most likely at the surface of the cell is preferred and often required. Also in this event harvesting of cells expressing the IGS3 polypeptide and the relevant RAMP prior to use in screening assays is required. If the IGS3 polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered. Membranes expressing the IGS3 polypeptide can be recovered by methods that are well known to a person skilled in the art. In general, such methods include harvesting of the cells expressing the IGS3

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polypeptide and homogenization of the cells by a method such as, but not limited to, pottering. The membranes may be recovered by washing the suspension one or several times.

IGS3 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well-known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

This invention also relates to the use of IGS3 polynucleotides for use as diagnostic reagents. Detection of a mutated form of the IGS3 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of IGS3. Also in this event co-expression of relevant receptor activity modifying proteins can be required to obtain diagnostic assays of desired quality. Individuals carrying mutations in the IGS3 gene may be detected at the DNA level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled IGS3 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers et al., Science (1985) 230:1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton et al., Proc. Natl. Acad. Sci. USA (1985) 85: 4397-4401. In another embodiment, an array of oligonucleotide probes comprising the IGS3 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions

in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee et al., Science, Vol 274, pp 610-613 (1996)).

5 The diagnostic assays offer a process for diagnosing or determining a susceptibility to among other things the Diseases as mentioned above, through detection of mutation in the IGS3 gene by the methods described.

10 In addition, among other things, the Diseases as mentioned above can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of the IGS3 polypeptide or IGS3 mRNA.

15 Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an IGS3, in a sample derived from a host are well known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

20 In another aspect, the present invention relates to a diagnostic kit for among other things the Diseases or susceptibility to one of the Diseases as mentioned above.

The kit may comprise:

- (a) an IGS3 polynucleotide, preferably the nucleotide sequence of SEQ ID NO:1, or a fragment thereof; and/or
- (b) a nucleotide sequence complementary to that of (a); and/or
- 25 (c) an IGS3 polypeptide, preferably the polypeptide of SEQ ID NO:2, or a fragment thereof; and/or
- (d) an antibody to an IGS3 polypeptide, preferably to the polypeptide of SEQ ID NO: 2; and/or
- 30 (e) a RAMP polypeptide required for the relevant biological or antigenic properties of an IGS3 polypeptide.

It will be appreciated that in any such kit, (a), (b), (c) (d) or (e) may comprise a substantial component.

Chromosome Assays

The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location
5 on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in
10 Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected
15 individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Antibodies

20 The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them if required together with relevant RAMP's, may also be used as immunogens to produce antibodies immunospecific for the IGS3 polypeptides. The term "immunospecific" means that the antibodies have substantial greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

25 Antibodies generated against the IGS3 polypeptides may be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique, which provides antibodies produced by continuous cell line cultures, may be used. Examples
30 include the hybridoma technique (Kohler, G. and Milstein, C., Nature (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., Immunology Today (1983) 4:72) and the EBV-hybridoma technique (Cole et al., MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

35 The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against IGS3 polypeptides as such, or against IGS3 polypeptide-RAMP complexes, may also be employed to treat among other things the Diseases as mentioned above.

5 **Animals**

Another aspect of the invention relates to non-human animal-based systems which act as models for disorders arising from aberrant expression or activity of IGS3. Non-human animal-based model systems may also be used to further characterize the activity of the IGS3 gene.
10 Such systems may be utilized as part of screening strategies designed to identify compounds which are capable to treat IGS3 based disorders such as among other things the Diseases as mentioned above.

In this way the animal-based models may be used to identify pharmaceutical compounds, therapies and interventions which may be effective in treating disorders of aberrant expression
15 or activity of IGS3. In addition such animal models may be used to determine the LD₅₀ and the ED₅₀ in animal subjects. These data may be used to determine the *in vivo* efficacy of potential IGS3 disorder treatments.

Animal-based model systems of IGS3 based disorders, based on aberrant IGS3 expression or
20 activity, may include both non-recombinant animals as well as recombinantly engineered transgenic animals.

Animal models for IGS3 disorders may include, for example, genetic models. Animal models exhibiting IGS3 based disorder-like symptoms may be engineered by utilizing, for
25 example, IGS3 sequences such as those described, above, in conjunction with techniques for producing transgenic animals that are well known to persons skilled in the art. For example, IGS3 sequences may be introduced into, and overexpressed and/or misexpressed in, the genome of the animal of interest, or, if endogenous IGS3 sequences are present, they may either be overexpressed, misexpressed, or, alternatively, may be disrupted in order to
30 underexpress or inactivate IGS3 gene expression.

In order to overexpress or misexpress a IGS3 gene sequence, the coding portion of the IGS3 gene sequence may be ligated to a regulatory sequence which is capable of driving high level gene expression or expression in a cell type in which the gene is not normally expressed in
35 the animal type of interest. Such regulatory regions will be well known to those skilled in the art, and may be utilized in the absence of undue experimentation.

For underexpression of an endogenous IGS3 gene sequence, such a sequence may be isolated and engineered such that when reintroduced into the genome of the animal of interest, the endogenous IGS3 gene alleles will be inactivated, or "knocked-out". Preferably, the engineered IGS3 gene sequence is introduced via gene targeting such that the endogenous
5 IGS3 sequence is disrupted upon integration of the engineered IGS3 gene sequence into the animal's genome.

Animals of any species, including, but not limited to, mice, rats, rabbits, squirrels, guinea-pigs, pigs, micro-pigs, goats, and non-human primates, e.g., baboons, monkeys, and
10 chimpanzees may be used to generate animal models of IGS3 related disorders.

Any technique known in the art may be used to introduce a IGS3 transgene into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to pronuclear microinjection (Hoppe, P.C. and Wagner, T.E., 1989, U.S. Pat. No. 4,873,191);
15 retrovirus mediated gene transfer into germ lines (van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152, 1985); gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321, 1989,); electroporation of embryos (Lo, Mol. Cell. Biol. 3:1803-1814, 1983); and sperm-mediated gene transfer (Lavitrano et al., Cell 57:717-723, 1989); etc. For a review of such techniques, see Gordon, Transgenic Animals, Intl. Rev. Cytol. 115:171-229, 1989.

20

The present invention provides for transgenic animals that carry the IGS3 transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, i.e., mosaic animals. (See, for example, techniques described by Jakobovits, Curr. Biol. 4:761-763, 1994) The transgene may be integrated as a single transgene or in concatamers, e.g., head-to-
25 head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko, M. et al., Proc. Natl. Acad. Sci. USA 89:6232-6236, 1992).

The regulatory sequences required for such a cell-type specific activation will depend
30 upon the particular cell type of interest, and will be apparent to those of skill in the art.

When it is desired that the IGS3 transgene be integrated into the chromosomal site of the endogenous IGS3 gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous IGS3
35 gene of interest (e.g., nucleotide sequences of the mouse IGS3 gene) are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of, the nucleotide sequence of the endogenous IGS3 gene or gene allele.

The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene of interest in only that cell type, by following, for example, the teaching of Gu et al. (Gu, H. et al., Science 265:103-106, 1994). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be
5 apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant IGS3 gene and protein may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to assay
10 whether integration of the transgene has taken place. The level of mRNA expression of the IGS3 transgene in the tissues of the transgenic animals may also be assessed using techniques which include but are not limited to Northern blot analysis of tissue samples obtained from the animal, in situ hybridization analysis, and RT-PCR. Samples of target gene-expressing tissue, may also be evaluated immunocytochemically using antibodies specific for the target gene transgene
15 product of interest. The IGS3 transgenic animals that express IGS3 gene mRNA or IGS3 transgene peptide (detected immunocytochemically, using antibodies directed against target gene product epitopes) at easily detectable levels may then be further evaluated to identify those animals which display characteristic IGS3 based disorder symptoms.

20 Once IGS3 transgenic founder animals are produced (i.e., those animals which express IGS3 proteins in cells or tissues of interest, and which, preferably, exhibit symptoms of IGS3 based disorders), they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include but are not limited to:
25 lines; inbreeding of separate lines in order to produce compound IGS3 transgenics that express the IGS3 transgene of interest at higher levels because of the effects of additive expression of each IGS3 transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the possible need for screening of animals by DNA analysis; crossing of separate homozygous lines
30 to produce compound heterozygous or homozygous lines; breeding animals to different inbred genetic backgrounds so as to examine effects of modifying alleles on expression of the IGS3 transgene and the development of IGS3-like symptoms. One such approach is to cross the IGS3 transgenic founder animals with a wild type strain to produce an F1 generation that exhibits IGS3 related disorder-like symptoms, such as those described above. The F1 generation may
35 then be inbred in order to develop a homozygous line, if it is found that homozygous target gene transgenic animals are viable.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises administering to (for example by inoculation) the
5 mammal the IGS3 polypeptide, or a fragment thereof, if required together with a RAMP polypeptide, adequate to produce antibody and/or T cell immune response to protect said animal from among other things one of the Diseases as mentioned above.

Yet another aspect of the invention relates to a method of inducing immunological
10 response in a mammal which comprises delivering the IGS3 polypeptide via a vector directing expression of the IGS3 polynucleotide in vivo in order to induce such an immunological response to produce antibody to protect said animal from diseases.

A further aspect of the invention relates to an immunological/vaccine formulation
15 (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to an IGS3 polypeptide wherein the composition comprises an IGS3 polypeptide or IGS3 gene. Such immunological/vaccine formulations (compositions) may be either therapeutic immunological/vaccine formulations or prophylactic immunological/vaccine formulations. The vaccine formulation may further comprise a suitable carrier. Since the IGS3
20 polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile
25 suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other
30 systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Screening Assays

35 The IGS3 polypeptide of the present invention may be employed in a screening process for compounds which bind the receptor and which activate (agonists) or inhibit activation of (antagonists) the receptor polypeptide of the present invention. Thus, polypeptides of the

invention may also be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics.

5

IGS3 polypeptides are responsible for biological functions, including pathologies. Accordingly, it is desirable to find compounds and drugs which stimulate IGS3 on the one hand and which can inhibit the function of IGS3 on the other hand. In general, agonists are employed for therapeutic and prophylactic purposes for such conditions as among other things the

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Diseases as mentioned above.

Antagonists may be employed for a variety of therapeutic and prophylactic purposes for such conditions as among other things the Diseases as mentioned above.

15

In general, such screening procedures involve producing appropriate cells, which express the receptor polypeptide of the present invention on the surface thereof and, if essential co-expression of RAMP's at the surface thereof. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*. Cells expressing the receptor (or cell membrane containing the expressed receptor) are then contacted with a test compound to observe binding, or stimulation or inhibition

20

of a functional response.

One screening technique includes the use of cells which express the receptor of this invention (for example, transfected CHO cells) in a system which measures extracellular pH, intracellular pH, or intracellular calcium changes caused by receptor activation. In this technique, compounds may be contacted with cells expressing the receptor polypeptide of the present invention. A second messenger response, e.g., signal transduction, pH changes, or changes in calcium level, is then measured to determine whether the potential compound activates or inhibits the receptor.

25

Another method involves screening for receptor inhibitors by determining modulation of a receptor-mediated signal, such as cAMP accumulation and/or adenylate cyclase activity. Such a method involves transfecting an eukaryotic cell with the receptor of this invention to express the receptor on the cell surface. The cell is then exposed to an agonist to the receptor of this invention in the presence of a potential antagonist. If the potential antagonist binds the receptor, and thus inhibits receptor binding, the agonist-mediated signal will be modulated.

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Another method for detecting agonists or antagonists for the receptor of the present invention is the yeast-based technology as described in U.S. Patent 5,482,835.

5 The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the receptor is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor. Further, these assays may test whether the candidate compound results in a signal generated by activation of the receptor, using detection systems appropriate to the cells bearing the receptor at their surfaces. Inhibitors of activation are generally assayed in the presence of a known
10 agonist and the effect on activation by the agonist by the presence of the candidate compound is observed.

Further, the assays may simply comprise the steps of mixing a candidate compound with a solution containing an IGS3 polypeptide to form a mixture, measuring the IGS3 activity in the
15 mixture, and comparing the IGS3 activity of the mixture to a standard.

The IGS3 cDNA, protein and antibodies to the protein may also be used to configure assays for detecting the effect of added compounds on the production of IGS3 mRNA and protein in cells. For example, an ELISA may be constructed for measuring secreted or cell
20 associated levels of IGS3 protein using monoclonal and polyclonal antibodies by standard methods known in the art, and this can be used to discover agents which may inhibit or enhance the production of IGS3 (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues. Standard methods for conducting screening assays are well known in the art.

25 Examples of potential IGS3 antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligand of the IGS3, e.g., a fragment of the ligand, or small molecules which bind to the receptor but do not elicit a response, so that the activity of the receptor is prevented.

30 Thus in another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, etc. for IGS3 polypeptides; or compounds which decrease, increase and/or otherwise enhance the production of IGS3 polypeptides, which comprises:

- 35
- (a) an IGS3 polypeptide, preferably that of SEQ ID NO:2;
 - (b) a recombinant cell expressing an IGS3 polypeptide, preferably that of SEQ ID NO:2;
 - (c) a cell membrane expressing an IGS3 polypeptide, preferably that of SEQ ID NO:2; or
 - (d) antibody to an IGS3 polypeptide, preferably that of SEQ ID NO: 2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

5 **Prophylactic and Therapeutic Methods**

This invention provides methods of treating abnormal conditions related to both an excess of and insufficient amounts of IGS3 activity.

10 If the activity of IGS3 is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit activation by blocking binding of ligands to the IGS3, or by inhibiting interaction with a RAMP polypeptide or a second signal, and thereby alleviating the abnormal condition.

15 In another approach, soluble forms of IGS3 polypeptides still capable of binding the ligand in competition with endogenous IGS3 may be administered. Typical embodiments of such competitors comprise fragments of the IGS3 polypeptide.

20 In still another approach, expression of the gene encoding endogenous IGS3 can be inhibited using expression-blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, J Neurochem (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, Florida USA (1988). Alternatively, oligonucleotides, 25 which form triple helices with the gene, can be supplied. See, for example, Lee et al., Nucleic Acids Res (1979) 6:3073; Cooney et al., Science (1988) 241:456; Dervan et al, Science (1991) 251:1360. These oligomers can be administered per se or the relevant oligomers can be expressed in vivo. Synthetic antisense or triplex oligonucleotides may comprise modified bases or modified backbones. Examples of the latter include methylphosphonate, phosphorothioate or 30 peptide nucleic acid backbones. Such backbones are incorporated in the antisense or triplex oligonucleotide in order to provide protection from degradation by nucleases and are well known in the art. Antisense and triplex molecules synthesized with these or other modified backbones also form part of the present invention.

35 In addition, expression of the IGS3 polypeptide may be prevented by using ribozymes specific to the IGS3 mRNA sequence. Ribozymes are catalytically active RNAs that can be

natural or synthetic (see for example Usman, N. et al., Curr. Opin. Struct. Biol (1996) 6(4), 527-33.) Synthetic ribozymes can be designed to specifically cleave IGS3 mRNAs at selected positions thereby preventing translation of the IGS3 mRNAs into functional polypeptide. Ribozymes may be synthesized with a natural ribose phosphate backbone and natural bases, as
5 normally found in RNA molecules. Alternatively the ribozymes may be synthesized with non-natural backbones to provide protection from ribonuclease degradation, for example, 2'-O-methyl RNA, and may contain modified bases.

For treating abnormal conditions related to an under-expression of IGS3 and its activity,
10 several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates IGS3, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous
15 production of IGS3 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral
20 particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells in vivo and expression of the polypeptide in vivo. For overview of gene therapy, see Chapter 20, Gene Therapy and other Molecular Genetic-based Therapeutic Approaches, (and references cited therein) in Human Molecular Genetics, Strachan T. and Read A.P., BIOS Scientific Publishers Ltd (1996).

25 Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

Formulation and Administration

30

Peptides, such as the soluble form of IGS3 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Formulation should suit the
35 mode of administration, and is well within the skill of the art. The invention further relates to

pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

5 Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration
10 include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible.

The dosage range required depends on the choice of peptide or compound, the route of
15 administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages are in the range of 0.1-100 $\mu\text{g/kg}$ of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than
20 administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example,
25 cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide ex vivo, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

The following examples are only intended to further illustrate the invention in more detail,
30 and therefore these examples are not deemed to restrict the scope of the invention in any way.

**EXAMPLE 1. THE CLONING OF GENOMIC DNA ENCODING A NOVEL G PROTEIN-
COUPLED RECEPTOR.****5 Example 1a. Homology PCR cloning of a genomic fragment encoding a novel G-protein
coupled receptor (GPCR).**

A PCR based homology cloning strategy was used to isolate partial genomic DNA
sequences encoding novel G-protein coupled receptors (GPCR). The following forward (F20)
10 and reverse (R42, R43) degenerate PCR primers were designed in conserved areas of the
neurotensin receptor gene family (Vita N. et al. [1993] Febs Lett. 317: 139-142; Vita N. et al.
[1998] Eur. J. Pharmacol. 360: 265-272) at the boundary of intracellular loop n°1 (I1) with
transmembrane domain 2 (TM2) and at the boundary of transmembrane domain 3 with
intracellular loop n°2 (TM3/I2) respectively:

15

F20 (I1/TM2):

5'-CTGCACTACCGGTGCTC(A or T)(G or C)(A,C,G or T)(C or T)T(A,C,G or T)GC -3'

(SEQ ID NO: 3)

20

R42 (TM3/I2):

5'-GGGTGGCAGATGGCCA(A or G)(A or G)(C or T)A(A,C,G or T)C(G or T)(C or T)TC(C or
Inosine)(C,G or T)

(SEQ ID NO: 4)

25

R43 (TM3/I2):

5'-GTGGCAGATGGCCAGGCAGCG(A or G)TC(A,C,G or T)(A or G)C(A or G)CT(A,G or T) -3'

(SEQ ID NO: 5)

In order to suppress amplification of known members of the neurotensin receptor family, the 3'
30 ultimate nucleotide position of primers R42 and R43 was chosen in such a way that it was either
not complementary to the corresponding position of the human NTR1 cDNA (R42) or to the
corresponding position of both NTR1 and NTR2 cDNA (R43).

The primary PCR reaction was carried out in a 60µl volume and contained 100 ng
human genomic DNA (Clontech), 6 µl GeneAmp™ 10 x PCR buffer II (100mM Tris-HCl pH 8.3;
35 500 mM KCl, Perkin Elmer), 3.6 µl 25 mM MgCl₂, 0.36µl dNTPs (25mM of each dNTP), 1.5 units
AmpliTaq Gold™ polymerase (Perkin Elmer) and 30 pmoles of each of the degenerated forward
(F20) and reverse primer (R42). Reaction tubes were heated at 95°C for 10 min and then

subjected to 35 cycles of denaturation (95°C, 1 min), annealing (55°C, 2 min) and extension (72°C, 3min). Finally reaction tubes were heated for 10 min at 72°C.

For the semi-nested PCR reaction 1 µl of a 1/50 dilution of the primary PCR reaction was used as a template using the degenerate forward and reverse primers F20 and R43 respectively. The semi-nested PCR reaction was carried out under the same conditions as the primary PCR reaction.

Semi-nested PCR reaction products were size fractionated on an agarose gel and stained with ethidium bromide. A fragment of \pm 220 bp was identified, purified from gel using the Qiaex-II™ purification kit (Qiagen) and ligated into the pGEM-T plasmid according to the procedure recommended by the supplier (pGEM-T kit, Promega). The recombinant plasmids thus produced were used to transform competent *E. coli* SURE™ 2 bacteria (Stratagene). Transformed cells were plated on LB agar plates containing ampicillin (100 µg/ml). Plasmid DNA was purified from mini-cultures of individual colonies using a Qiagen-tip 20 miniprep kit (Qiagen). DNA sequencing reactions were carried out on the purified plasmid DNA with the ABI Prism™ BigDye™ Terminator Cycle Sequencing Ready Reaction kit (PE-ABI), using insert-flanking. Sequencing reaction products were purified via EtOH/NaOAc precipitation and analysed on an ABI 377 automated sequencer.

A computer-assisted homology search of the insert sequence of clone HNT1370 against public domain sequence databanks (Blastn; Altschul S.F. et al. [1997], Nucleic Acids Res. 25:3389-3402) revealed strong indications that it encoded (part of) a novel member of the GPCR family. Although HNT1370 had been cloned from a \pm 220 bp fragment the insert size was only \pm 130 bp as a result of a cloning artefact. We refer to this novel GPCR sequence as IGS3.

Table 3: Overview of oligo primers used.

SEQ ID NO: 3	F20: 5'-CTGCACTACACGTGCTC(A or T)(G or C)(A,C,G or T)(C or T)T(A,C,G or T)GC -3'
SEQ ID NO: 4	R42: 5'-GGGTGGCAGATGGCCA(A or G)(A or G)(C or T)A(A,C,G or T)C(G or T)(C or T)TC(C or Inosine)(C,G or T)
SEQ ID NO: 5	R43: 5'-GTGGCAGATGGCCAGGCAGCG(A or G)TC(A,C,G or T)(A or G)C(A or G)CT(A,G or T) -3'
SEQ ID NO: 6	IP11969: 5'GGGGCCGACTTCCTCTTCCTCTGCTTCC-3'
SEQ ID NO: 7	IP12008: 5'-GCAAGGTAGGCACAGGTCATCACAGTGG-3'
SEQ ID NO: 8	IP12936: 5'-ATAAGCTTCTCCCTGGCCCTTAATAAATGAC-3'
SEQ ID NO: 9	IP12937: 5'-AGGAATTCAGACAGACAGGGGCAAAGTTG-3'

Example 1b. Cloning of genomic DNA fragments containing the complete IGS3 coding sequence.

5 The complete coding sequence of IGS3 was obtained via hybridization screening of a human genomic library. A human genomic DNA library (Clontech #HL1067j), constructed in the lambda EMBL3 SP6/T7 phage vector was screened by hybridization using an IGS3 specific probe. This probe was derived from a 130 bp PCR fragment amplified from the HNT1355 plasmid (which contained an identical insert as HNT1370) using IGS3 specific primers IP11969
10 (SEQ ID NO: 6) and IP12008 (SEQ ID NO: 7) (Fig.1). The 130 bp fragment was purified from gel using the Qiaex-II™ purification kit (Qiagen) and radiolabelled via random primed incorporation of [α -³²P]dCTP to a specific activity of > 10⁹ cpm/μg using the Prime-It II kit™ (Stratagene) according to the instructions provided by the supplier. Approximately 550,000 plaques were screened with the 130 bp probe according to the Lambda Library User Manual of Clontech
15 (PT1010-1). Three positive clones (λ-IGS3.1, λ-IGS3.3 and λ-IGS3.5) were plaque-purified and recombinant phage DNA was prepared from small-scale liquid cultures as described by Maniatis et al. (Sambrook, J. et al. Molecular Cloning: A Laboratory Manual Second Edition [1989], CSH Laboratory Press).

Sequence analysis of the recombinant phage DNA using IGS3 specific primers showed
20 that the inserts of all 3 lambda clones contained a long open reading frame encoding a novel putative (intron-less) GPCR of 330 amino acids (the postulated start of translation was preceded by an in-frame stop codon). The IGS3 coding sequence was subcloned into a plasmid vector after PCR amplification. PCR reactions were carried out on the isolated λ-IGS3.1, λ-IGS3.3 and λ-IGS3.5 phage DNA (500 ng) with the IP12936 (SEQ ID NO: 8) and IP12937 (SEQ ID NO: 9)
25 oligonucleotide primers using the Expand™ High Fidelity PCR system (Boehringer). PCR reaction tubes were heated at 95°C for 2 min and then subjected to 35 cycles of denaturation (95°C, 30 sec), annealing (58°C, 30 sec) and extension (72°C, 1 min). There was a final elongation step at 72°C (10 min). A ± 1,200 bp PCR product was purified from gel and ligated into the pGEM-T vector. The recombinant DNA was then used to transform E.coli bacterial strain
30 DH5αF'. This yielded bacterial clones HB4971, HB4972 (both subcloned from λ-IGS3.1), HB4973 and HB4974 (both subcloned from λ-IGS3.3) and HB4975 and HB4976 (both subcloned from λ-IGS3.5). The inserts of all plasmid clones were completely sequenced. A meld of all sequence data yielded a consensus sequence, which confirmed the existence of a long open reading frame of 330 amino acids that encoded a putative novel GPCR receptor (IGS3)
35 (Fig.1). The consensus cDNA and protein sequence of IGS3 are presented here as IGS3DNA (SEQ ID NO: 1) and IGS3PROT (SEQ ID NO: 2) respectively. Homology searches of DNA

databanks with the IGS3DNA sequence showed one EST sequence (accession n° AF003828) which partially overlapped with IGS3DNA at the 3' end (Fig.1).

- 5 The bacterial strain harboring plasmid HNT4971 (containing the IGS3DNA sequence) was recloned after replating on LB agar plates containing 100 µg ampicillin/ml and deposited both in the Innogenetics N.V. strain list (ICCG4319) and at the "Centraalbureau voor Schimmelculturen (CBS)" in Baarn, The Netherlands (accession n° 102196). Plasmid DNA was prepared from the recloned isolate and the insert was resequenced and found to be identical to the IGS3DNA sequence.

PCT

Original (for SUBMISSION) - printed on 15.09.2000 04:00:52 PM

0-1	Form - PCT/RO/134 (EASY) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)	
0-1-1	Prepared using	PCT-EASY Version 2.90 (updated 15.12.1999)
0-2	International Application No.	
0-3	Applicant's or agent's file reference	SPW99.07
1	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
1-1	page	33
1-2	line	3>
1-3	Identification of Deposit	
1-3-1	Name of depositary institution	Centraalbureau voor Schimmelcultures
1-3-2	Address of depositary institution	Oosterstraat 1, Postbus 273, NL-3740 AG Baarn, Netherlands
1-3-3	Date of deposit	15 September 1999 (15.09.1999)
1-3-4	Accession Number	CBS 102196
1-4	Additional Indications	NONE
1-5	Designated States for Which Indications are Made	all designated States
1-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE

FOR RECEIVING OFFICE USE ONLY

0-4	This form was received with the international application: (yes or no)	
0-4-1	Authorized officer	

FOR INTERNATIONAL BUREAU USE ONLY

0-5	This form was received by the international Bureau on:	
0-5-1	Authorized officer	

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Duphar International Research B.V.
Postbus 900
1380 DA WEESP
The Netherlands

name and address of depositor

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM

Identification reference given by the
DEPOSITOR:
E. coli DH5 alpha F' pGEM-ThIGS3 ICCG 4319

Accession number given by the
INTERNATIONAL DEPOSITARY AUTHORITY:
CBS 102196

II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION

The microorganism identified under I above was accompanied by:

- ☒ a scientific description
☐ a proposed taxonomic designation
(mark with a cross where applicable)

III. RECEIPT AND ACCEPTANCE

This International Depositary accepts the microorganism identified under I above, which
received by it on 15-09-99 (date dd-mm-yy of the original deposit) 1

IV. RECEIPT OF REQUEST FOR CONVERSION

The microorganism identified under I above was received by this International Depositary
Authority on not applicable (date dd-mm-yy of the original deposit) and a
request to convert the original deposit to a deposit under the Budapest Treaty was received
by it on not applicable (date dd-mm-yy of receipt of request for conversion)

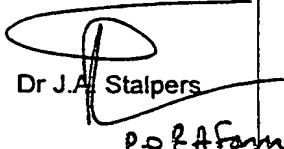
V. INTERNATIONAL DEPOSITARY AUTHORITY

Name: Centraalbureau voor Schimmelcultures

Address: Oosterstraat 1
P.O. Box 273
3740 AG BAARN
The Netherlands

Signature(s) of person(s) having the power
to represent the International Depositary
Authority or of authorized official(s):


Mrs F.B. Snippe-Claus


Dr J.A. Stalpers
p.o. 2 A. van

Date (dd-mm-yy): 17-09-99

1 Where Rule 6.4(d) applies, such date is the date on which the status of international
depositary authority was acquired.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Duphar International Research B.V.
Postbus 900
1380 DA WEESP
The Netherlands

*name and address of the party to whom the
viability statement is issued*

VIABILITY STATEMENT



issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified on the following page

I. DEPOSITOR	II. IDENTIFICATION OF THE MICROORGANISM
Name: Duphar International Research B.V.	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: CBS 102196
Address: Postbus 900 1380 DA WEESP The Netherlands	Date (dd-mm-yy) of the deposit or of the transfer: 1 15-09-99
III. VIABILITY STATEMENT	
The viability of the microorganism identified under II above was tested on 17-09-99 ² . On that date (dd-mm-yy), the said microorganism was <input checked="checked" type="checkbox"/> ³ viable <input type="checkbox"/> ³ no longer viable	

¹ Indicate the date of the original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).

² In the cases referred to in Rule 10.2(a)(ii) and (iii), refer to the most recent viability test.

³ Mark with a cross the applicable box.

IV. CONDITIONS UNDER WHICH THE VIABILITY HAS BEEN PERFORMED 4	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
<p>Name: Centraalbureau voor Schimmelcultures</p> <p>Address: Oosterstraat 1 P.O. Box 273 3740 AG BAARN The Netherlands</p>	<p>Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):</p> <p> Mrs F.B. Snippe-Claus Date (dd-mm-yy): - 17-09-99</p> <p> Dr V.A. Statpers P.O. RASamson</p>

⁴ Fill in if the information has been requested and if the results of the test were negative.